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# Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2

Tracking no: BLD-2021-012217R1

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**Abstract:**
Vaccination using the adenoviral vector COVID-19 vaccine ChAdOx1 nCoV-19 (AstraZeneca) has been associated with rare vaccine-induced immune thrombotic thrombocytopenia (VITT). Affected patients test strongly positive in PF4/polyanion enzyme immunoassays (EIAs) and serum-induced platelet activation is maximal in the presence of PF4. We determined the frequency of anti-PF4/polyanion antibodies in healthy vaccinees and assessed whether PF4/polyanion EIA-positive sera exhibit platelet-activating properties after vaccination with ChAdOx1 nCoV-19 (n=138) or BNT162b2 (BioNTech/Pfizer; n=143). In total, 19 of 281 participants tested positive for anti-PF4/polyanion antibodies post-vaccination (All: 6.8% [95%CI, 4.4-10.3]; BNT162b2: 5.6% [95%CI, 2.9-10.7]; ChAdOx1 nCoV-19: 8.0% [95%CI, 4.5-13.7%]). Optical densities were mostly low (between 0.5-1.0 units; reference range, <0.50) and none of the PF4/polyanion EIA-positive samples induced platelet activation in the presence of PF4. We conclude that positive PF4/polyanion EIAs can occur after SARS-CoV-2 vaccination with both mRNA- and adenoviral vector-based vaccines, but the majority of these antibodies likely have minor (if any) clinical relevance. Accordingly, low-titer positive PF4/polyanion EIA results should be interpreted with caution when screening asymptomatic individuals after vaccination against Covid-19. Pathogenic platelet-activating antibodies that cause VITT do not occur commonly following vaccination.

**Conflict of interest:** COI declared - see note

**COI notes:** Dr. Greinacher reports grants and non-financial support from Aspen, Boehringer Ingelheim, MSD, Bristol Myers Squibb (BMS), Paringenix, Bayer Healthcare, Gore Inc., Rovi, Sagent, Biomarin/Prosensa, personal fees from Aspen, Boehringer Ingelheim, MSD, Macopharma, BMS, Chromatec, Instrumentation Laboratory, non-financial support from Boehringer Ingelheim, Portola, Ergomed, GTH e.V. outside the submitted work. Dr. Thiele reports grants from Deutsche Forschungsgemeinschaft, during the conduct of the study; personal fees and other from Bristol Myers Squibb, personal fees and other from Pfizer, personal fees from Bayer, personal fees and other from Chugai Pharma, other from Novo Nordisk, personal fees from Novartis, other from Daichii Sankyo, outside the submitted work. Dr. Warkentin reports personal fees from Aspen Global, Ergomed, Instrumentation Laboratory, and Octapharma, all of which are outside of the submitted work. Dr. Selleng received personal fees from Aspen Germany and travel support from Sobi, outside the submitted work. Dr. Bröker reports personal fees from Pfizer, Novartis and GSK outside the submitted work.

**Preprint server:** No;

**Author contributions and disclosures:** TT, TEW and AG designed the study. TT, LU, SH and LS performed the study. KB and NOH are principal investigators who conducted SeCo. BMB is principal investigator of AICOVI. SOK and CS are coordinators of the UMG vaccination program. TT, LS, TEW and AG analysed the data. TT, TEW, KS , KA and AG wrote the manuscript. All authors read and approved the final version of this manuscript.

**Non-author contributions and disclosures:** Yes; SeCo Study Team: E. Baufeld, M. Beer, J. Bohnert, N. Endlich, M. Groschup, E.A. Idelevich, M. Lerch, T. C. Mettenleiter, M. Nauck, C. Rutscher, W. Schlumberger, M. Tzvetkov, H. Völzke, K. Zimmermann, M. Zygmunt AICOVI Study Team: Kilian A. Wietschel, Kevin Reppschläger, Elmer Antileo, Chiara A. Drechsler, Erika Friebe Vaccination Team: Ali Aghdassi, Marèn Fricke, Holger Lode, Astrid Radau, Christine Rutscher, Anna Seidlein Transfusion Medicine Platelet

Lab and Blood Service: Ulrike Strobel, Carmen Freyer, Ricarda Raschke, Ines Warnig, Jessica Fuhrmann, Katrin Stein, Kathrin Kunze We acknowledge Katja Schulz for her commitment and contribution as study nurse in the SeCo study and we are grateful to the technicians of the diagnostics team of the Friedrich Loeffler-Institute of Medical Microbiology. We thank all study participants for their support. Funding: COVIDPROTECT, Ministerium für Wirtschaft, Arbeit und Gesundheit of the Federal State Mecklenburg-Vorpommern. The study has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - Projektnummer 374031971 - TRR 240.

**Agreement to Share Publication-Related Data and Data Sharing Statement:** Data are available via direct contact by emails to the corresponding author

**Clinical trial registration information (if any):** This study was performed as a substudy of two ongoing clinical studies: the "Screening for COVID-19 and Monitoring of Serological Responses to SARS-CoV-2 in Healthcare Workers study" (SeCo, NCT 04370119); and the "Adaptive Immune Response against Corona Virus Vaccination study" (AlCOVI, NCT 04826770)

**Brief Report**

# Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2.

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**Key points:**

- Low-titer PF4/polyanion antibodies occur after vaccination with ChAdOx1 nCoV-19 and BNT162b2
- These PF4/polyanion antibodies do not activate platelets and may have little relevance for the diagnosis of VITT


**Summary**

Vaccination using the adenoviral vector COVID-19 vaccine ChAdOx1 nCoV-19 (AstraZeneca) has been associated with rare vaccine-induced immune thrombotic thrombocytopenia (VITT). Affected patients test strongly positive in PF4/polyanion enzyme immunoassays (EIAs) and serum-induced platelet activation is maximal in the presence of PF4. We determined the frequency of anti-PF4/polyanion antibodies in healthy vaccinees and assessed whether PF4/polyanion EIA-positive sera exhibit platelet-activating properties after vaccination with ChAdOx1 nCoV-19 (n=138) or BNT162b2 (BioNTech/Pfizer; n=143). In total, 19 of 281 participants tested positive for anti-PF4/polyanion antibodies post-vaccination (All: 6.8% [95%CI, 4.4-10.3]; BNT162b2: 5.6% [95%CI, 2.9-10.7]; ChAdOx1 nCoV-19: 8.0% [95%CI, 4.5-13.7%]). Optical densities were mostly low (between 0.5-1.0 units; reference range, <0.50) and none of the PF4/polyanion EIA-positive samples induced platelet activation in the presence of PF4. We conclude that positive PF4/polyanion EIAs can occur after SARS-CoV-2 vaccination with both mRNA- and adenoviral vector-based vaccines, but the majority of these antibodies likely have minor (if any) clinical relevance. Accordingly, low-titer positive PF4/polyanion EIA results should be interpreted with caution when screening asymptomatic individuals after vaccination against Covid-19. Pathogenic platelet-activating antibodies that cause VITT do not occur commonly following vaccination.

## Introduction

Vaccines against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) are a cornerstone in controlling the SARS-CoV-2 pandemic[1-4]. By March 2021, the European Medical Agency approved four vaccines to prevent symptomatic Covid-19, all encoding the spike protein antigen of SARS-CoV-2: two mRNA-based vaccines BNT162b2 (BioNTech/Pfizer) and mRNA1273 (Moderna); and two recombinant vector-based vaccines: adenovirus type 26 vector Covid-19 Vaccine Janssen (Johnson&Johnson), and the recombinant chimpanzee adenoviral vector vaccine ChAdOx1 nCoV-19 (AstraZeneca).

We and others have recently described vaccine-induced immune thrombotic thrombocytopenia (VITT) associated with ChAdOx1 nCoV-19 vaccination. VITT presents between 5 to 20 days following vaccination with thrombocytopenia (median platelet count, ~20 × $10^9$/L); unusual and severe thromboembolic events such as cerebral venous sinus thrombosis and splanchnic vein thrombosis; often signs of disseminated intravascular coagulation (DIC); and the presence of IgG antibodies that react strongly in platelet factor 4 (PF4)/polyanion enzyme-immunoassays (EIAs) and activate platelets in the presence of PF4[5-7]. Hence, VITT shares features with autoimmune heparin-induced thrombocytopenia (HIT) including severe thrombocytopenia, DIC, and heparin-independent platelet-activating properties without previous heparin exposure[8].VITT has to date only been described as a rare complication after vaccination with the adenoviral vector-based vaccines ChAdOx1 nCoV-19 and Covid-19 Vaccine Janssen[9].

Antibodies of VITT patients bind to PF4 alone, but also to PF4 in PF4/heparin complexes. If VITT is suspected, a screening test for PF4/polyanion antibodies is recommended[5,10] to confirm the presence of high-titer anti-PF4 antibodies. It is well known from HIT that anti-PF4/polyanion antibodies among heparin-exposed patients are heterogeneous, with only a minority of IgG exhibiting strong heparin-dependent platelet-activating properties. Further, preexisting B-cells exist which can produce anti-PF4 antibodies. These B-cells are present even in cord blood[11]. But activation of these B-cells requires an appropriate antigen and additional co-signals. The frequency of these antibodies is especially high in patients after major surgery indicating that tissue trauma and/or inflammation[12] provide an important co-signal for induction of anti-PF4 antibody production. After vaccination against Covid-19,

inflammatory responses including fever, chills and headaches are frequently reported. This raises the question how frequently platelet-activating anti-PF4/polyanion IgG occur after vaccination against Covid-19 and whether there is a difference between vector-based and mRNA-based vaccines.

## Materials and methods

*Study design*

Vaccination of health care workers was performed between January and March 2021 in an institutional program of the University Medicine of Greifswald (UMG). Subjects received either two doses of BNT162b2 (Comirnaty®, BioNTech/Pfizer) with an interval of 21-28 days between doses; or one dose of ChadOx1 nCoV-19 (Vaxzevria®, AstraZeneca AB).

This study was performed as a substudy of two ongoing clinical studies: the "Screening for COVID-19 and Monitoring of Serological Responses to SARS-CoV-2 in Healthcare Workers study" (SeCo, NCT 04370119); and the "Adaptive Immune Response against Corona Virus Vaccination study" (AICOVI, NCT 04826770). Both studies are conducted at the UMG and assess the incidence of seroconversion against SARS-CoV-2 among health care workers during the pandemic and/or due to vaccination. Participants gave written and informed consent; the local Ethics Committee approved both studies (BB 068/20 and BB 001/21).

Blood samples from recipients of BNT162b2 were analyzed after the first and the second vaccine dose (SeCo: variable time points; AICOVI: day 0 [before vaccination], 7 and 14 days after two doses 28 days apart). Samples from ChAdOx1 nCoV-19 recipients were analysed before and after the first vaccine dose (SeCo: variable time points; AICOVI: day 0 [before vaccination] and day 7). From a subset of SeCo-participants, a pre-vaccination sample was available. A history of SARS-CoV-2 infection was assessed by questionnaire in both studies. In SeCo, a nasopharyngeal swab for SARS-CoV-2 PCR was obtained at study entry. For each participant, date of vaccination, type of vaccine, date of blood sampling, age, and sex were analyzed. Samples were tested for anti-PF4/polyanion antibodies. In case of a positive test result, samples were tested for heparin and PF4-dependent platelet-activating antibodies.

*Anti-PF4/polyanion antibody testing and platelet activation assay*

An in-house IgG-specific PF4/polyanion EIA[13] was used to screen for antibodies recognizing PF4 and PF4/heparin complexes[14]. Positive results were given in optical density (OD) units, as follows (reference range <0.50): weak reaction, 0.5 to ≤1.0 units; strong reaction >1.0 units). Samples testing positive by PF4/heparin EIA were assessed for platelet-activating antibodies by a washed platelet activation assay in the presence of PF4 (10µg/mL)[5].

## Results and discussion

In total, 281 vaccinees were assessed, of whom 143 (50.9%) received BNT162b2, and 138 (49.1%) the ChAdOx1 nCoV-19 vaccine; 73.3% were female. Platelet counts were not followed in this study; however, none of the 281 study participants developed thrombosis or clinically evident VITT.

After vaccination, sera of 19 subjects tested positive for anti-PF4/polyanion antibodies corresponding to an overall frequency of 6.8% (95%CI, 4.4-10.3). After BNT162b2 vaccination, sera of 8/143 participants tested positive (5.6%; 95%CI 2.9-10.7); and after ChAdOx1 nCoV-19 vaccine, 11/138 (8.0%; 95%CI 4.5-13.7%) tested positive ($p>0.05$). Eighteen of 19 antibody-positive vaccinees showed ODs between 0.5-1.0 units (weak reaction); however, one sample (recipient of ChAdOx1 nCov-19 vaccine) showed an OD >2.0 (Figure 1).

For 11/19 anti-PF4/polyanion IgG positive individuals, pre-vaccination samples were available (4 received BNT162b, 7 received ChAdOx1 nCoV-19): Here, 7/11 sera tested already positive before vaccination. However, four individuals showed “seroconversion”, as they tested negative before vaccination and positive after vaccination (ODs before/after: ChAdOx1 nCoV-19: 0.22/0.89 and 0.50/0.77; BNT162b2 0.05/0.75 and 0.41/0.80; Figure 1C-F).

Importantly, none of the sera testing EIA-positive for anti-PF4/polyanion antibodies could reproducibly activate platelets in a platelet activation assay in the presence of added PF4. Two sera tested initially weakly/borderline positive, but negative on repeat testing. Of note, platelet-activating antibodies could not be excluded in the two

positive participants of the AICOVI study, because samples were collected in EDTA. However, ODs were low (OD: 0.7-0.8).

The frequency of positive anti-PF4/polyanion IgG tests in our study appears higher than observed in healthy blood donors. Hursting, et al., found a frequency of 6.6% seropositive anti-PF4/polyanion samples among 3.795 blood donors[15], but used a test detecting IgG, IgA and IgM. Our group found no PF4/polyanion IgG-positive individuals among 923 blood donors[16]. However, blood donors are a preselected group less likely to have underlying inflammatory conditions. In contrast, patients with strong inflammation have a higher likelihood for testing positive for anti-PF4/polyanion IgG: Among ICU patients, 6.3% tested positive at admission and 17.2% after 10 days of intensive care[17]. Additionally, the rate of PF4/polyanion IgG seroconversion increases with the severity of trauma[12]. Our data suggest that vaccination against COVID-19 leads to anti-PF4 seroconversion in a few subjects and that this may occur after vaccination with either ChAdOx1 nCoV-19 or BNT162b2 as part of the inflammatory response.

A positive anti-PF4/polyanion EIA alone is not sufficient to diagnose VITT, especially if the reactivity strength is low. In contrast to the sera from healthy vaccinees analyzed here, sera from patients with clinically-overt VITT are strongly-positive by anti-PF4/polyanion EIA (typically ODs>2), and cause strong platelet activation in a washed platelet activation assay in the presence of PF4.[5,6] However, high-titer, platelet-activating anti-PF4 antibodies, as observed in VITT, appear to be uncommon in vaccinees (< 0.5%). Therefore, PF4/polyanion EIAs results need to be judged in the clinical context, particularly with occurrence of thrombocytopenia and/or thrombosis in a typical time window of 5-20 days following vaccination[10]. A positive PF4/polyanion EIA result should be interpreted with caution in clinically asymptomatic individuals recently vaccinated against SARS-CoV-2.

**Acknowledgements:**

SeCo Study Team: E. Baufeld, M. Beer, J. Bohnert, N. Endlich, M. Groschup, E.A. Idelevich, M. Lerch, T. C. Mettenleiter, M. Nauck, C. Rutscher, W. Schlumberger, M. Tzvetkov, H. Völzke, K. Zimmermann, M. Zygmunt

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We acknowledge Katja Schulz for her commitment and contribution as study nurse in the SeCo study and we are grateful to the technicians of the diagnostics team of the Friedrich Loeffler-Institute of Medical Microbiology. We thank all study participants for their support.

**Funding:** COVIDPROTECT, Ministerium für Wirtschaft, Arbeit und Gesundheit of the Federal State Mecklenburg-Vorpommern. The study has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 374031971 – TRR 240.

**Authorship Contributions:** TT, TEW and AG designed the study. TT, LU, SH and LS performed the study. KB and NOH are principal investigators who conducted SeCo. BMB is principal investigator of AICOVI. SOK and CS are coordinators of the UMG vaccination program. TT, LS, TEW and AG analysed the data. TT, TEW and AG wrote the manuscript. All authors read and approved the final version of this manuscript.

**Disclosure of Conflicts of Interest**

Dr. Greinacher reports grants from Deutsche Forschungsgemeinschaft and non-financial support from Aspen, Boehringer Ingelheim, MSD, Bristol Myers Squibb (BMS), Paringenix, Bayer Healthcare, Gore Inc., Rovi, Sagent, Biomarin/Prosensa, personal fees from Aspen, Boehringer Ingelheim, MSD, Macopharma, BMS, Chromatec, Instrumentation Laboratory, non-financial support from Boehringer Ingelheim, Portola, Ergomed, GTH e.V. outside the submitted work.

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**Table 1: Frequency of anti-PF4/polyanion IgG in vaccinated individuals**

| **Vaccine** | BNT162b2 | ChAdOx1 nCoV-19 | Total |
|---|---|---|---|
| Total (%) | 143 (50.9) | 138 (49.1) | 281 (100) |
| Age (median) | 43 | 45 | 44 |
| Female (%) | 107 (74.8) | 99 (71.7) | 206 (73.3) |
| Postvaccination PF4/polyanion EIA+ (%) | 8 (5.5) | 11 (8.0) | 19 (6.8) |
| | | | |
| SeCo substudy (n %) | 111 (47.4) | 123 (52.6) | 234 (100) |
| Postvaccination PF4/polyanion EIA+ (%) | 7# (6.3) | 10# (8.1) | 17# (7.3) |
| Baseline samples available | 3 | 6 | 9 |
| Baseline PF4/polyanion EIA+ (%)** | 2 | 4 | 6 |
| | | | |
| AICOVI substudy (n %) | 32 (68.1) | 15 (31.9) | 47 (100) |
| Postvaccination PF4/polyanion EIA+ (%) | 1 (3.1) | 1 (6.7) | 2 (4.3) |
| Baseline PF4/polyanion EIA+ (%) | 0 (0) | 1 (6.7) | 1 (2.1) |

#8 participants tested positive after vaccination had no available baseline sample (4 with BNT162b2 and 4 with CHADOX1 nCoV-19)

** tested were only participants with positive PF4/polyanion EIA after vaccination who had an available baseline sample before vaccination.

## Figure Legends

**Figure 1:** Anti-PF4/polyanion IgG in relation to the time point of vaccination (day 0) with BNT162b2 and ChAdOx1 nCoV-19. An optical density (OD) of >0.5 units was considered positive (grey shaded area: reference range OD<0.5). SeCo substudy**:** **(A)** Sera of 111 individuals who were vaccinated twice with BNT162b2; **(B)** 123 individuals vaccinated once with ChAdOx1 nCoV-19. **(C)** Seropositive subjects with available baseline samples vaccinated with BNT162b2 and **(D)** with ChAdOx1 nCoV-19. Baseline samples were taken at a median of 261 days before BNT162b2- and at a median of 169 days before ChAdOx1 nCoV-19 vaccination. Subjects with increasing ODs after vaccination have uninterrupted lines.

AICOVI-substudy: **(E)** Sera of 47 participants tested at pre-vaccination baseline (day 0) and at days 7 and 14 after two doses of BNT162b2 (first dose, day 0; second dose, day 28) and **(F)** one dose of ChAdOx1 nCoV-19 (day 0). Subjects with increasing ODs after vaccination are shown in colored triangles.